XIII.—*On the Agamic Reproduction of a Species of* Chironomus, *and its Development from the Unfecundated Egg.* By Oscar von Grimm.

[Concluded from p. 45.]

III. *The Development of the Embryo in the Unfecundated Ovum.*

The embryonic development of *Chironomus* in the fertilized ovum has already frequently been investigated and described by various observers, such as Kölliker, Kupfer, Weismann, Metschnikow*, and Melnikow; but the development from the unfecundated ovum, as, indeed, even the ovum itself, has hitherto been unknown.

We shall see hereafter that the development of the embryo from the fecundated and unfecundated ovum perfectly agrees, so that we might content ourselves with describing the points of divergence between our investigations and those of other observers, if it were not that we intended publishing a more detailed memoir on the development of the histological elements and organs. But, as I have already made some investigations in this direction, and will, as soon as possible, lay the results of this work before the reader, I regard it as necessary to give here a short summary of the course of development, especially as, in some cases, I have arrived at different results from Weismann, who, however, has most completely investigated the embryology of *Chironomus*.

I must, however, remark that I shall here rarely refer to authors, as I regard this chapter as a preface to my future work.

As we already know that in *Chironomus* the second, asexually produced generation is developed from ova, we need not discuss the opinion of Wagner† and Meinert‡ upon the genesis of the Cecidomyid larvæ from the fatty body of the parent larva (to which, moreover, Meinert ascribes peculiar properties), since the supposition of Pagenstecher§ that the larvæ originate independently of the fatty body has been proved by

* Prof. Metschnikow's most recent investigation upon the embryology of *Chironomus* is unfortunately only partially known to me, as it is not yet published.

† "Beitrag zur Lehre von der Fortpflanzung der Insectenlarven," Zeitschr. für wiss. Zool. 1863, Bd. xiii. p. 522.

‡ "Weitere Erläuterungen über die von Prof. Wagner beschriebene Insectenlarve," &c. Zeitschr. für wiss. Zool. Bd. xiv. p. 395.

§ "Die ungeschlechtliche Vermehrung der Fliegenlarven," Zeitschr. für wiss. Zool. Bd. xiv. p. 410.

the investigations of Leuckart*, Ganin†, and Metschnikow‡, as they have detected the ovaries in the Cecidomyid larvæ, and studied the genesis of the larvæ from the ova. Indeed Prof. Wagner himself afterwards recognized his error.

When a certain number of ova have attained maturity within the organism of the parent pupa, the pupa expels them, through the above-mentioned orifices situated in the penultimate ventral segment, in the form of two cords consisting of a homogeneous mass, in each of which there are from 20 to 50 ova. These newly laid ova are elongated oval, egg-shaped, with an obtuse and an acute pole. In the former the head of the embryo is afterwards situated; and it is therefore indicated as the cephalic pole, whilst the opposite one is named the caudal pole. The ova are 0·22 millim. in length and 0·09 millim. in breadth. They are filled with a brownish-yellow vitellus, which contains a quantity of rather large oil-drops. At the upper or cephalic pole the chorion forms an impression, on the margins of which is affixed an extremely elegant lobule (Pl. III. fig. 12, *l*) which formerly united the ova with one another. Whether there is a micropylar orifice in this impression I do not know.

In the preceding chapter we have seen that in the ovum when still incompletely developed but already half filled with the yelk, and changed from the conical to the oval form, the germinal vesicle was already present, although the germinal spot could not be found in it. From this circumstance alone we might come to the conclusion that the germinal vesicle exists in the perfectly developed and deposited ovum; nevertheless all my endeavours to discover the germinal vesicle were without result, although I resorted to the most various reagents and methods of investigation. Notwithstanding this, however, I was firmly convinced that the so-called germ-nuclei are developed in the ovum of *Chironomus*, as indeed of all insects, by the division of the germinal vesicle. We know that the germinal vesicles have originated by the division of the nucleus of the ovarian tube. This circumstance alone leads us to assume that the germinal vesicle also divides and thus produces the germ-nuclei, but not that it is destroyed; for in agamic reproduction the fecundation which ought to cause this destruction of the germinal vesicle is wanting. And in fact, after I had in vain examined many hundred ova with this

* "Die ungeschlechtliche Fortpflanzung der Cecidomyienlarven," Arch. für Naturg. 1865, p. 290.

† Zapiski Imp. Ak. Nauk, 1865, vol. vii. p. 46.

‡ Zhurnal Mni. Nar. Pr. 1865: Embryologische Studien an Insecten, p. 20.

view, I was so fortunate a few days ago as to detect, quite unexpectedly, what I had so long sought in vain. I laid the abdomen of a pupa containing well-formed ova in glycerine, and in the course of a few days, when I remembered this preparation, I examined the ova under the microscope. My delight may be imagined when I saw very distinctly the germinal vesicle in a series of ova (fig. 12, *g v*), and with them an ovum with the germinal vesicle engaged in division. The germinal vesicle was 0·045 millim. in diameter. Its division takes place in a direction transverse to the ovum. These two objects seem to prove prefectly that the germinal vesicle by no means disappears, but by dividing becomes converted into the germ-nuclei. But the circumstance that so many admirable observers (as Weismann, for instance) could not find it, and thought themselves compelled to assume a free formation of the germ-nuclei, was probably caused by the opacity of the yelk, and the difficulty of investigation dependent thereupon.

We may now therefore assume that the union between the germ-nuclei and the germinal vesicle, and also between the different generations, actually exists, and therefore that *omnis cellula e cellula.*

The first alteration in the deposited ovum consists in the contraction of the contents in the direction of the longitudinal axis of the ovum. In consequence of this contraction a polar space is formed in each end of the ovum, of which the lower one, or that in the caudal pole, is larger than the opposite one.

We then observe an alteration in the periphery of the yelk: there is formed here a homogeneous, limpid blastema-layer, the so-called blastema of the blastoderm (*Keimhautblastem*, Weism.), which appears to be thickest in the region of the inferior pole of the ovum. This blastema is nothing but a homogeneous mass, which has separated from the yelk; it is therefore a part of the vitellus, and may probably be regarded as the formative vitellus of insects, whilst the yelk enclosed in this functions as the nutritive vitellus. Soon after the separation of the formative vitellus a germ-nucleus makes its appearance in the inferior pole of the ovum, and, surrounded by a portion of the formative vitellus, passes as the so-called polar cell into the inferior polar space; here the membraneless cell divides into two cells, each of which again divides, so that we finally obtain four polar cells. Frequently, however, the nucleus of the first polar cell divides while still lying in the layer of formative vitellus, so that two polar cells appear at once in the polar space. During the appearance of the first polar cell we see many germ-nuclei, formed by the division of the germinal vesicle, pass from the nutritive vitellus into the layer of formative vitellus, at the same time continually dividing. Here each germ-nucleus is surrounded by a layer of the formative vitellus, so that the germ-nuclei are converted into cell-nuclei, and the layer of formative vitellus becomes a cell-layer, which may be described as the blastoderm. The development of the blastoderm commences, however, in the lower pole of the ovum ; that is to say, the germ-nuclei make their appearance in the inferior polar space sooner than in the superior. The cells of the blastoderm, the nuclei of which, as is well known, are strongly refractive, divide in the direction of the radii of the ovum, so that the blastoderm soon appears as a layer of elongated, cylindrical cells. After the completion of the longitudinal division of these cells they divide transversely, so that from the originally one-layered blastoderm we get a two-layered structure. The cells of the lower blastodermic layer now formed continue dividing in the same direction, so that this layer soon appears as a multistratified cell-mass, the outer layer retaining its original character and its cells not dividing. In consequence of this the boundary between these two blastodermic layers is easily recognized.

Having now briefly described the formation of the blastoderm, we venture to raise the question whether this is not identical with the so-called segmentation of the vitellus in other animals ? We know that two kinds of ova are distinguished among animals,—those which only contain the formative vitellus being designated *holoblastic*, and the others, which have both the formative and the nutritive vitellus, *meroblastic* ova. The insect-ovum, however, possesses at first only one sort of vitellus, which subsequently divides into the nutritive and the formative vitellus. Hence the insect-ovum may be regarded first as holoblastic, and afterwards as meroblastic. The insect-egg, therefore, unites these two kinds of ova with each other, representing a transition form. We know, further, that the segmentation of the ovum is either total, as in the holoblastic ova, or partial, as in the meroblastic ova. Both consist in the division of the first *sphere of segmentation* (in which there is a nucleus with a nucleolar corpuscle, which must probably be regarded as the germinal vesicle*) into a great number of small spheres ; this process is most properly interpreted by Kölliker † as "a kind of cell-multiplication process." Does not this take place also in insects ? Have we not seen the division of the germinal

* According to Johannes Müller, Gegenbaur, and Leydig.

† Entwickelungsgeschichte des Menschen und der höheren Thiere, p. 30.

vesicle into germ-nuclei, and the envelopment of these by the formative vitellus, and the formation of the blastodermic cells, which certainly appear to be the analogues of the spherules of segmentation? Is it, then, possible to overlook the identity between the process of segmentation and the formation of the blastoderm? It is true that in the insect-ovum numerous cells (representing spherules of segmentation) are formed at once, after the germinal vesicle, which lies in the nutritive vitellus, has divided into numerous germ-nuclei; but this is caused by the circumstance that the insect-ovum is neither holoblastic nor meroblastic; and the later separation of the formative vitellus is also a consequence of this anomaly in the development of the spherules of segmentation as we may call it.

Claparède*, Leuckart†, and Metschnikow‡ have, indeed, already expressed the opinion, in opposition to Weismann, that the formation of the blastoderm is a process analogous to segmentation; but to me these two processes appear to be perfectly identical, and I have therefore ventured to dwell at some length upon this question.

The inner blastodermic layer, which has become converted, in consequence of the transverse division of its cells, into a finely cellular, many-layered cell-mass, now thickens, but chiefly in the region of the convex side of the ovum.

In consequence of the formation and thickening of the blastoderm, the size of the total contents of the ovum increases, and they now occupy the polar spaces. The four polar cells consequently become immersed in the blastodermic layer, from which, however, they shine forth very distinctly. Their metamorphosis into the ovaries we have already witnessed, and therefore we refer to them no further.

In consequence of the continuous division of the cells the blastoderm has become converted into a finely cellular, strongly refractive mass surrounding the nutritive vitellus on all sides. Immediately after this we observe the formation of the *germinal streak*, which is formed by a thickening of the inner blastoderm in the ventral surface of the ovum. The development of the germinal streak consists first of all in the formation of the so-called caudal pad. At the same time the primitive caudal furrow appears; but this soon disappears, having no further consequences. The form of the caudal pad may be best recognized from the form of the nutritive vitellus.

* Recherches sur l'évolution des Araignées.

† "Die Fortpflanzung und Entwicklung der Pupiparen," Abhandl. naturf. Gesellsch. zu Halle, iv. p. 210.

‡ Embryologische Studien an Insecten, pp. 93–95.

On examining the ovum at this period from the ventral surface, it is easy to perceive that the nutritive vitellus has acquired a form different from the original one; it has become attenuated at each end and gibbous in the middle, and at the same time much lighter in its posterior half, where the caudal pad is situated, which is caused by its less thickness, because here, as has been said, the pad of the germinal streak has consumed it. Then, whilst the caudal pad is constantly enlarging, the opposite cephalic ridge is formed.

As the caudal pad becomes elongated, it ascends nearly to the middle of the ovum, *i. e.* approaches the cephalic end of the ovum. At this time there rises upon its dorsal surface a transverse elevation, the margin of which is turned towards the inferior pole of the ovum. This elevation, growing, becomes converted into a fold which covers half the caudal pad. This is the so-called *caudal fold.*

As regards the whole blastodermic mass, this thickens in the ventral side of the head, and becomes attenuated in the opposite or dorsal side. At the time when the dorsal blastoderm has attained its minimum thickness, a curved dark streak is to be seen, from the dorsal surface of the ovum, upon the nutritive vitelline mass; its dark colour is due to the more considerable vitelline mass here placed, or to the blastodermic layer being most attenuated here. This streak very soon acquires more distinct limits and a still darker colour. At this time occurs the so-called bursting of the blastoderm, upon which Weismann has established that type of development which he designates by the name of "regmagene."

During this bursting, the caudal fold has already grown far downward, and at the moment of bursting it embraces the caudal pad in its whole thickness.

The whole embryo moves 180° upon its longitudinal axis; so that its belly comes to lie in the flat side of the ovum, and its back in the convex one.

Somewhat later we observe a diminution of thickness in the nutritive vitelline mass lying in the cephalic extremity, *i. e.* a thickening of the cephalic portion of the germinal streak, the formation of the cephalic hood. Here a fold is then formed, the margin of which is directed towards the superior pole of the ovum. It grows much more rapidly than the caudal fold, so that the margins of these two folds reach the middle of the embryo at the same time. As their margins grow together, they now form only one fold, which covers the whole dorsal surface of the embryo. With the growth of this fold (*i. e.* the embryonal envelope) the place of origin of the caudal fold has moved far up, and it soon occupies the free space formed by the cleaving of the blastoderm, contracting the vitellus constantly more and more.

The embryonal envelope is at this time bent round the extremities of the embryo, and now shows only one small foramen, the margins of which may be very well seen from the ventral side of the embryo. The vertical laminæ are also already to be seen.

A little after the stage of development just described, a longitudinal furrow, the *median vitelline ridge* of Weismann, becomes perceptible on the inner surface of the germinal streak, dividing the whole germinal streak into two *germinal pads*. This is accompanied by the complete closure of the embryonal envelope, so that now the oval foramen is no longer to be seen. The walls of this embryonal envelope, which have even previously begun to divide, now, after the union of their free margins, form two envelopes separated from each other. One of these envelopes, which lies immediately beneath the chorion, forms a complete capsule, in which the embryo lies freely. This capsule, which has been formed from the outer wall of the embryonal envelope, is Metschnikow's *amnion insectorum*, Kupfer's *embryonal envelope*, and Brandt's *external embryonal envelope*; the other wall of the entire embryonal envelope forms Metschnikow's *covering lamella* (Deckblatt), Kupfer's *folded lamella*, and Brandt's *inner embryonal envelope**.

Somewhat later the germinal pads become segmented in the middle of the length of the ovum; the three pairs of primitive jaws are formed. During this process the inner embryonal envelope is ruptured at the head, and the fore part of the head shows itself through the ruptured place. While this rupture of the inner embryonal envelope enlarges, the germinal pads also increase in length, so that the furrow separating them from each other now reaches only to the fore part of the head; hence the so-called cephalic pad may now be distinguished. The vertical plates, which must be regarded as the foundation of the inner embryonal envelope, give off the primitive antennæ. Then we also observe the further development of the jaws, but especially of the mandibles, which now appear as pointed irregular segments. They have their points turned upwards.

Now also occurs the constriction of the cephalic segments, the three pairs of jaws moving towards the upper half of the ovum. The abdominal furrow, also, which is not unlike those seen at the commencement of the development, now becomes visible.

* The two together form the folded lamella of Weismann.

By the growth of the vertical plates, the fore part of the head is bent forwards. The vitelline streak situated in the fore part of the head becomes diminished, and finally disappears altogether. The inner embryonal envelope now covers only the dorsal surface of the embryo, having removed by its rupture, which commenced at the head, from the ventral surface and the two extremities of the embryo.

The constriction of the cephalic parts goes on, and at this time also the vertical plates separate by a transverse furrow from the caudal pad, by which means the head acquires a distinctly limited form. Immediately after the limitation of the head, the formation of the ventral segments commences, first three and then the rest of the segments being formed.

During the constriction of the cephalic parts, the embryo for the second time revolves round its longitudinal axis, and again by 180°, so that it gets to lie again in its original position; *i. e.* its ventral surface moves again into the convex side of the ovum.

The further development of the head consists in the mandibles occupying the place of the antennæ, whilst the latter move on to the sides of the fore part of the head. At this time the intestinal tube is seen.

After the constriction of the vertical plates from the germinal pads is completed, the embryo draws itself together, which, indeed, has really caused this constriction. The contraction is indicated by the downward movement of the abdominal extremity. At the same time commences the curvature of the mandibles and the lateral movement of the first pair of maxillæ, which become converted into the palpi; the second pair of maxillæ, however, become united by their median margins. On the lower surface of the thoracic segment a transverse fold is formed; and this is afterwards converted into the anterior legs.

Finally, we see that the abdominal extremity has passed entirely into the inferior pole of the ovum. The anal orifice, formed previously by inversion, is now clearly visible. The walls of the intestinal tube consist of a layer of large, oval cells.

In consequence of the constriction of the head and the downward movement of the abdomen (or contraction of the embryo) the vitelline mass, of course, passes outward and lies immediately beneath the inner embryonal envelope. During the whole contraction of the embryo, the lateral walls formed by the rupture of the blastoderm grow into thin processes, which gradually overgrow the vitelline mass, and finally close the back of the embryo, as was quite correctly observed by

Melnikow. The anal orifice is now bounded by four elevations, which subsequently become developed into the finger-like processes. The posterior pair of legs is formed by a furrow, which divides the last ventral segment from beneath into two elevations. Three claws appear upon the two pairs of feet. At the same period the larval eyes and eleven consecutive ganglia are to be seen, occupying the lower space of the ventral cavity. The yelk-sac has now become immersed, and no longer appears as a separate structure; its walls have become considerably thicker.

Lastly, the antennæ and then the palpi become segmented. The body of the embryo becomes much elongated; and as it lies in the envelope of the ovum, it draws this out, so that the ovum now measures 0·27 millim.; the envelope, however, is still so strong that it bears this pressure; and thus the gradually elongating larva is compelled at first to lie in irregular folds, and then to twist itself into a spiral of 2½ turns. Even at the commencement of this process, movements of the larva are perceptible; but when the larva has rolled itself up, the contractions become very lively. It also now works with its fore feet and mandibles, which are now perfectly developed and have become brown. Contractions are also observable in the walls of the stomach.

The ovum now bursts; and the larva, which is 0·47 millim. in length, creeps about in the cavity of the homogeneous cord until, in the course of a few hours, it breaks through this membrane also.

The whole process of development, from the deposition of the ovum to the exclusion of the larva, lasts, in spring, from eighty to ninety hours.

I now conclude this short description of the embryonic development of the species of *Chironomus* under investigation, and reserve it for some future day to describe the development of the histological elements and organs, and also to discuss the question of the lamellar theory of insect-development.

EXPLANATION OF PLATE III.

Fig. 1. The string of ova laid by the pupa: *a*, homogeneous mass.

Fig. 2. The newly hatched larva; its stomach is still filled with the remains of the vitellus.

Fig. 3. A larva, 3 millims. in length, in which the pupa is developing, some of its parts being already visible, such as the tracheæ, eyes, wings, and legs. *Fig.* 3 *a*, antenna of the same larva, strongly magnified.

Fig. 4. Ovigerous pupa; its wings are bent downwards, and only one of them is to be seen.

In figs. 2, 3, & 4, the lettering is as follows:—*a*, antennæ;

e, eye; *p e*, pupal eye; *f*, finger-shaped processes; *ff*, fore foot; *hf*, hinder foot; *pf*, pupal feet; *s*, stomach; *pv*, proventriculus; *mx*, maxillæ; *md*, mandibles; *p*, palpi; *ms*, muscles; *tr*, tracheæ; *fb*, fatty bodies; *tf*, terminal filament; *o*, ova; *s b*, stigmatic branchiæ; *c b*, chitinous band; *g o*, genital orifice; *a o*, anal orifice; *pn*, pronotum; *w*, wing.

Fig. 5. A portion of an ovum. The blastoderm has acquired several layers; and in its mass are the polar cells, the diameter of which is 0·012 millim.

Fig. 6. The same, rather later; the polar cells have separated into two groups: *a*, furrow.

Fig. 7. The developing ovary removed from a larva contracted in folds; diameter 0·031 millim.: *e c*, embryonal cells; *n*, nucleus of the ovarian tube.

Fig. 8. An ovary removed from a perfectly developed larva; six embryonic ovarian tubes are visible in it, containing nuclei; diameter 0·057 millim.: *e c*, embryonal cells; *o t*, ovarian tubes; *n*, nucleus.

Fig. 9. Ovarian tube from a young larva: *v c*, vitelligenous cells; *ep*, epithelium; *n*, nucleus.

Fig. 10. Part of an ovarian tube (magn. 475 diams.). The left side of this figure represents the object seen on the surface, and the right side the optical transverse section. The ovum (*o*) will soon be detached. The vitelligenous cells (*v c*) are only indicated, and are too small; *t f*, terminal filament; *t c*, terminal chamber; *ep*, epithelial cells; *ep'*, epithelial cells as seen at the surface; *g c*, germ-chamber; *o d*, oil-drops; *g v*, germinal vesicle; *g s*, germinal spot; *v c*, vitelligenous cells; *v*, vitellus. Diameter of the ovum (*o*) 0·085 millim., of the germinal vesicle 0·0432 millim., of the vitelligenous cells 0·0250 millim., of the epithelial cells 0·0224 millim., of the germinal spot 0·0078 millim.; length of the germ-chamber 0·044 millim.; length of the terminal chamber 0·922 millim., diameter of its nuclei 0·0085 millim.; thickness of the terminal filament 0·0060 millim.; diameter of the oil-drops 0·0048 millim.

Fig. 11. Portion of an ovarian tube at the moment of its division into chambers, the contents being already divided, and the nucleus in course of division; magn. 630 diams. The lettering as in fig. 10.

Fig. 12. A perfectly developed ovum in which the germinal vesicle is visible, preserved in glycerine; diameter of the germinal vesicle (*g v*) 0·045 millim.: *l*, lobule surrounding the micropyle.

XIV.—*Notes on the* Berardius *of New Zealand.* By Dr. J. E. Gray, F.R.S. &c.

Dr. Hector kindly sent me an early impression of Dr. Knox's and his own paper on the *Ziphiidæ*, illustrated with five plates, which is to be published in the third volume of the 'Transactions of the New-Zealand Institute.' It contains a figure of the animal of *Berardius*, various parts of the skeleton, and the details of two skulls. From it I give the following character to the animal of this genus, which was previously known only